STUDIES ON GREGARINA BLATTARUM
WITH PARTICULAR REFERENCE TO ENCYSTMENT, SYNGAMY AND SPORE DEVELOPMENT

BY

VICTOR SPRAGUE

B. Ed., Southern Illinois State Normal University, 1932

M. S., University of Illinois, 1938

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SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN ZOOLOGY IN THE GRADUATE SCHOOL OF THE UNIVERSITY OF ILLINOIS, 1940

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STUDIES ON GREGARINA BLATTARUM WITH PARTICULAR REFERENCE TO ENCYSTMENT, SYNGAMY AND SPORE DEVELOPMENT

During the fall and winter of 1939 (September to the middle of December) 810 specimens of *Blatta orientalis* were examined for *Gregarina blattarum*. Only 5.3% were found to be infected and in each instance the number of parasites was small. At that time cysts were found but rarely.

In January and February of 1940 about 30% of a collection of roaches, which had been kept in the laboratory since the previous fall, were heavily infected. At that time great numbers of cysts were recovered daily from the feces. Some of the cysts were fed to the roaches in an attempt to maintain a high incidence of infection and others were used in these studies.

A few observations were made on the trophic stages of the parasite. Most of the sporadins occur in pairs regardless of their size or state of maturity. Although the two members of a pair are usually of the same size, the satellite is not infrequently smaller than the primite. The reciprocal relationship appears not to occur. Occasionally two small satellites, either alike or different in size, occur on one primite. These unusual associations encyst and develop in a normal manner.

The process of encystment was observed a number of times after pairs of mature sporadins were placed in fresh undiluted egg albumen on depression slides. The two individuals move forward and, at the same time, bend their bodies by contraction of the myonemes, which results in a rotating movement. With more bending of the bodies the two individuals come to lie side by side with the anterior end of each firmly attached to the posterior end of the other, thus forming an almost perfect sphere. While rotation continues the gregarines secrete a viscous substance which forms the cyst membrane of many layers. Before the membrane is completely formed the contraction of the bodies ceases and permits the two individuals to extend so that the young cyst changes from the shape of a sphere to that of a prolate spheroid. Cysts formed on a slide are more elongated than those formed within the host. The average ratio of the long to the short axis of fourteen of the former was found to be 1.76

while that of twenty-five cysts formed within the same host from which the other material was obtained was 1.22. These figures indicate that the amount of restriction as to space during the time when the myonemes are in a state of relaxation is an important factor in determining the shape of the cysts. Cysts formed in closely crowded surroundings tend to be more nearly spherical while those which are not confined during formation tend to be much elongated.

Further information on the shape of the cysts is obtained from a scatter diagram on which the long axes are plotted along the abscissa and the short axes on the ordinate. The resulting points fall about evenly on the two sides of a straight line thus showing a positive correlation between the long and short axes.

Having demonstrated that the variation in shape is about the same for all size groups it is possible to designate the sizes of cysts in terms of either of their two axes. A histogram showing the size distribution of 259 cysts indicates that encystment seldom occurs until the sporadin have attained a rather definite minimum size. At this size the majority encyst but some grow for an additional period of time before encystment, thus producing very large cysts. There are certain irregularities in the size distribution which cannot be explained with certainty at present.

When cysts are placed in a chamber, so constructed as to maintain the humidity within at the saturation point, they undergo a series of developmental changes which are easily observed. At an undetermined time gametes appear around the periphery of each individual within the cyst and are so arranged as to resemble a layer of columnar epithelial cells. This layer of gamates probably constitutes a cellular membrane which encloses the residual substance of the former sporadin. The residual mass, being in the colloidal state, consists of a number of materials suspended in an aqueous medium. The dispersed phase is retained within the cellular membrane while a portion of the dispersion medium passes through and forms an aqueous layer between the gamates and the cyst membrane. The gamates then separate and pass out into the aqueous layer. Thus there is no longer a boundary separating that layer from the rest of the cyst contents and soon a mixing of the entire contents

occurs, giving the cyst a uniform appearance throughout.

One day later the basal discs of the sporoducts are seen. Then, after two more days, the sporoducts are everted and the spores are discharged. The evertion of the sporoducts is explained in the following manner: Neutral red and brom-thymol blue indicate an acid reaction in the basal region of the sporoduct. Enzymes acting in an acid medium probably have a lytic effect on that portion of the cyst membrane which is in contact with the basal disc. The membrane becomes weakened at that point and the great pressure constantly exerted on the cyst contents by the elastic cyst membrane forces the sporoduct through the weakened area.

Immediately after the sporoducts are everted the spores are expelled in extremely long chains with as many as 10,000 individual spores in one continuous chain. The spore chains often arrange themselves in large coils containing many turns.

The mature spore is ellipsoidal or cylindrical in shape and with broadly rounded ends but is covered with a mucoid sheath which gives it a truncate appearance. The sheath is adhesive and holds the spores together in the chain. The size of the spore is approximately $8.7\mu \times 4.3\mu$.

A detailed study of the chromosome behavior during spore development was made from smear preparations. Cysts in the desired stage of development were selected from the moist chamber, crushed on cover-glasses, fixed in Schaudinn, Flemming or Zenker, stained with Heidenhain or Giemsa or treated by the Feulgen method. The best results were obtained with the Zenker-Heidenhain combination and with Schaudinn followed by Heidenhain, Feulgen or Giemsa.

The pregamic divisions were studied sufficiently to determine the number and appearance of the chromosomes. The chromosomes are most easily distinguished in the anaphase. Here they are seen to be three in number and have a characteristic arrangement. Two very small and slightly elongated chromosomes lie side by side in the anaphasic movement while the third, which is plainly longer, trails behind. On the basis of the appearance in the anaphase the two small chromosomes, which look

identical, are designated as 1 and 2 while the longer one is called 3. After an undetermined number of nuclear divisions the gametes appear around the periphery of the cyst and each has three chromosomes.

After the gamete separates from the residual mass it enters into a period of growth during which a large vesicle appears around the nucleus. Two gametes then come together in a characteristic manner and fuse. The characteristic position assumed by the two gametes during syngamy and differences in behavior of the pronuclei suggest psysiological anisogamy. One of the gametes is, therefore, designated as male and the other female.

A haploid set of three chromosomes is contributed to the synkaryon by each of the two pronuclei. The six chromosomes assume a characteristic polarized arrangement, which is common in the prophase of the first meiotic division of animals, and undergo synapsis and tetrad formation. Disjunction of tetrads 1 and 2 occurs before the breakdown of the nuclear membrane while disjunction of tetrad 3 occurs in the anaphase. Three dyads thus go to each daughter nucleus in the anaphase of the first zygotic division. The telophase reorganization results in two vesicular nuclei.

The two elements of each dyad are then separated in the second zygotic division, resulting in four vesicular nuclei each having a haploid complement of three chromosomes. The end of this division marks the end of the meiotic phenomena. The zygote is thus seen to be a meiocyte, the first division involving synapsis, tetrad formation and disjunction. In the second division the two chromatids in each dyad separate, and to each of the resulting four nuclei a haploid complement of univalent chromosomes, like that in the gamete, is restored. All succeeding stages are, therefore, haploid until the diploid condition again occurs in the zygote of the next generation.

The third zygotic division results in eight ring-shaped nuclei which transform themselves into long rods spirally arranged within the spore. These are the nuclei of the eight sporozoites.

The mature spores may be of two types with regard to the sporozoite nuclei. In some cysts the nuclei are in groups of four near the two ends of the spore and each nucleus has a slight

enlargement at the outer end and a greater enlargemnt at the inner end. In other cysts all of the eight sporozoite nuclei lie near the center of the spore and the smaller enlargement is directed toward the center while the greater enlargement is directed toward the end of the spore. This difference in the sporozoite nuclei and other discrepancies observed in the course of these studies suggest that two or more types of *Gregarina blattarum* may be involved.

Summary

1. In the fall and early winter of 1939 about 5.3% of 810 specimens of *Blatta orientalis* were found to harbor light infections of *Gregarina blattarum.*

2. In January and February of 1940 approximately 30% of a collection of roaches kept in the laboratory for several months were heavily infected.

3. Daily collections of cysts were made from feces in January and February of 1940. About 100 roaches yielded 200-400 cysts each day. The cysts were either fed to the roaches or used in further study.

4. The gregarines usually associate in pairs long before maturity. The members of a pair may be similar in size or the primite may be larger than the satellite. Sometimes two satellites are seen on one primite and cysts with three gametocytes are formed from them. These cysts develop in a normal manner.

5. The process of encystment is described.

6. The amount of restriction as to space during a certain critical period in encystment is an important factor in determining the shape of the cysts.

7. There is a positive correlation between the long and short axes of a sample of 259 cysts.

8. A histogram showing size distribution of cysts indicates a tendency for encystment to occur when a certain definite minimum size has been attained by the sporadins.

9. The normal course of development of cysts kept in a moist chamber is described.

10. The mechanism of sporoduct evertion is explained.

11. The spores are expelled in very long chains which often form large coils with many turns. As many as 10,000 individual

spores may occur in one continuous chain.

12. The mature spore is described.

13. A detailed study of the chromosome behavior during syngamy and spore development was made.

14. Three chromosomes are seen in the pregamic divisions and they are designated as 1, 2 and 3.

15. Because of a characteristic position assumed by the gametes during syngamy, and because of differences in behavior of the two pronuclei the gametes are considered to be physiologically anisogamous and are designated as male and female.

16. The zygote is a meiocyte which undergoes two meiotic divisions to produce four nuclei, each having the gametic number (3) of chromosomes.

17. A third zygotic division produces eight ring-shaped nuclei which transform into the long rod-shaped nuclei of the sporozoite.

18. Certain incongruities observed in the course of these studies suggest that more than one type of *Gregarina blattarum* may be involved.

VIII. VITA

Victor Sprague was born in Fayette County, Illinois, on November 16, 1908. He attended the elementary school at Carbondale, Illinois, and graduated from Carbondale Community High School in 1926. During a part of the year 1926-1927 he attended Union University, Jackson, Tennessee, then he entered Southern Illinois State Normal University, Carbondale, Illinois, from which he received the degree, Bachelor of Education, in 1932. Twelve weeks in the spring and summer of 1935 were spent at the University of California in Berkeley, California, doing graduate work in Protozoology. From 1937 to 1940 he was engaged in graduate study in Zoology at the University of Illinois, receiving the degree, Master of Science, in 1938. The summer of 1939 was spent in study and research at Chesapeake Biological Laboratory, Solomons Island, Maryland.

During the academic years 1933-1937 he was principal and teacher in the elementary schools at Royalton, Illinois. In 1936-1937 he taught Biology in the Royalton High School. He was employed during the Mid-Spring Term of 1936 to teach Vertebrate Embryology and Invertebrate Zoology at Southern Illinois State Normal University. While pursuing graduate study he was employed as assistant in Zoology at the University of Illinois.

He was elected in 1938 to active membership in Phi Sigma and associate membership in Sigma Xi, becoming an active member of Sigma Xi in 1940. He is also a member of the American Microscopical Society.

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